Stem Cells and Development

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Grafted neural progenitor cells persist in the injured site and differentiate neuronally in a rodent model of cardiac arrest-induced global brain ischemia

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Grafting of stem cells in ischemic brain injury

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Non-standard abbreviations:

CA1-3	*cornu ammonis* segments *1-3*
CC	*corpus callosum*
CXCR4:	CXC chemokine receptor 4
DG	dentate gyrus
FJ	Fluoro-Jade B
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
Iba1	ionized calcium binding adaptor molecule 1
NeuN	neuronal nuclear antigen
NPC	neural progenitor cell
ROSC	return of spontaneous circulation
SDF-1:	Stromal cell-derived factor 1
SOX2	SRY-box transcription factor 2

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Abstract

Hypoxic-ischemic brain injury is the leading cause of disability and death after successful resuscitation from cardiac arrest, and, to date, no specific treatment option is available to prevent subsequent neurofunctional impairments. The hippocampal *cornu ammonis* segment 1 (CA1) is one of the brain areas most affected by hypoxia, and its degeneration is correlated with memory deficits in patients and corresponding animal models. The aim of the present work was to evaluate the feasibility of neural progenitor cell (NPC) transplantation into the hippocampus in a refined rodent cardiac arrest model. Adult rats were subjected to 12 minutes of potassium-induced cardiac arrest and followed up to 6 weeks. Histological analysis showed extensive neuronal cell death specifically in the hippocampal CA1 segment, without any spontaneous regeneration. Neurofunctional assessment revealed transient memory deficits in ischemic animals compared to controls, detectable after 4, but not after 6 weeks. Using stereotactic surgery, embryonic NPCs were transplanted in a subset of animals 1 week after cardiac arrest and their survival, migration and differentiation were assessed histologically. Transplanted cells showed a higher persistence in the CA1 segment of animals after ischemia. Glia in the damaged CA1 segment expressed the chemotactic factor SDF-1, while transplanted NPCs expressed its receptor CXCR4, suggesting that the SDF-1/CXCR4 pathway, known to be involved in the migration of neural stem cells towards injured brain regions, directs the observed retention of cells in the damaged area. Using immunostaining, we could demonstrate that transplanted cells differentiated into mature neurons. In conclusion, our data document the survival, persistence in the injured area and neuronal differentiation of transplanted NPCs, and thus their potential to support brain regeneration after hypoxic-ischemic injury. This may represent an option worth further investigation in order to improve the outcome of patients after cardiac arrest.

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Introduction

The global incidence of out-of-hospital cardiac arrest is estimated to be 55 cases/100'000 persons/year, the average survival being 7% [1]. Among patients that are resuscitated, the leading cause of morbidity and mortality is hypoxic-ischemic brain damage [2], with half of the survivors left with cognitive deficits, in particular memory impairments [3,4]. Certain brain structures are especially vulnerable to ischemic injury, such as the pyramidal neurons in the *cornu ammonis* segment 1 (CA1) in the hippocampus [5-7]. Loss of these cells has been associated with memory deficits, in rodents [8] as well as in humans [9]. Currently, the only treatment recommendation after cardiac arrest that improves neurological outcome is targeted temperature management [10-13]. However, this practice will never completely prevent brain damage. During the last decades a plethora of compounds, targeting multiple stages of the hypoxic-ischemic injury cascade, have been tested as potential neuroprotective agents for brain damage following cardiac arrest. Although promising results were obtained in experimental studies, their benefits for patients with cardiac arrest could not be confirmed in clinical trials [14-16]. Thus, there is a strong need for novel therapeutic approaches. Cell-based therapies have gained increasing attention in the past few years as a possible strategy to mitigate brain damage. Numerous experimental studies and several clinical trials were conducted to evaluate the feasibility and safety of cell transplantation in stroke [17-19], but only few for the treatment of cardiac arrest [20-25]. Their results led to a cautious optimism that this therapeutic approach could reduce brain damage and improve neurological outcome [26].

In this study, we aimed to evaluate the feasibility of neural progenitor cell (NPC) transplantation after cardiac arrest. For this, we refined our rodent cardiac arrest model [27] by increasing the duration of no-flow time (from 10 to 12 minutes), aiming to induce more severe cognitive deficits, and describing long-term histological and neurofunctional outcomes.

Material and methods

Animals and experimental groups

All animal studies were approved by the Animal Care and Experimentation Committee of the Canton of Bern, Switzerland (licences BE 62/17 and BE64/17) and followed the Swiss national guidelines for the performance of animal experiments. 9 weeks old male Wistar

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rats were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and kept in individually ventilated cages (IVC) with controlled 12 hours light/dark cycles at 22±2°C. Food and water were provided *ad libitum*.

A total of 135 animals were included in the study. Animals underwent cardiac arrest (n=94) or served as controls (n=41). Histological analysis was assessed 1 week after surgery in 13 cardiac arrest and 7 control animals. Morris water maze was performed on two different subsets: 13 cardiac arrest and 10 control animals 4 weeks after surgery, and 13 cardiac arrest and 13 controls 6 weeks after surgery. After conclusion of the behavioural experiments, histological analysis was performed on all animals. The remaining 34 cardiac arrest and 11 control animals were enrolled in the second study, to evaluate the feasibility of stereotactic cell transplantation after cardiac arrest. Three animals did not survive stereotactic surgery, the remaining were histologically assessed after 1, 2 and 6 weeks.

Induction of cardiac arrest and resuscitation

Procedures are described briefly, details are provided in the supplementary material.

Cardiac arrest induction and resuscitation were performed as described previously [27]. Briefly, after induction of anaesthesia, animals were orotracheally intubated and catheters were inserted in the femoral artery for blood pressure monitoring and blood sampling, and in the jugular vein for drug administration. Cardiac arrest was induced by i.v. injection of potassium chloride (KCl) (Kalium Chloratum Sintetica, Sintetica SA) and esmolol (Esmolol-Orpha, OrPha Swiss GmbH). After 12 minutes rats were resuscitated by adrenaline and calcium injection and manual chest compressions. Control animals (sham group) underwent the same surgical interventions, without cardiac arrest induction.

Assessment of learning and memory function by Morris water maze

One week before learning and memory assessment, an open field test was conducted to test spontaneous locomotor activity of animals. Rats were placed in an open field arena (90x90x40 cm) and recorded during 5 minutes with the video tracking system Ethovision® XT-11 (Noldus Information Technology, Wageningen, Netherlands). Velocity and total distance moved by the animals were analysed as readout for general locomotor activity. Learning and memory performance were assessed by Morris water maze four or six weeks after cardiac arrest as previously described [28]. Swimming patterns of rats were recorded with the video tracking system Ethovision® XT-11 (Noldus Information Technology,

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Wageningen, Netherlands). The tank (1.8 m diameter) was filled with darkened water and the surface was virtually divided into four quadrants. An adjustable black platform (16 x 13 cm) was placed in the center of the first quadrant, 0.5 cm below the water surface, not visible for swimming rats. Three entry zones were marked outside the pool and several distal cues were placed on three walls surrounding the water maze. During 5 days, animals were trained with 4 trials per day to find the hidden platform in a fixed position. Rats were put into the water facing the tank wall at one of the entry zones, which was determined by randomization. If an animal found the platform within 90 seconds, it was allowed to stay on it for 20 seconds before continuing with the next trial. If the rat did not find the platform within 90 seconds, it was guided there by hand and was allowed to stay on it for 20 seconds. The parameters evaluated for training trials was latency to reach platform. On day 1, the first training trial was considered as “day 0”. Probe trials without the platform were conducted on the first before the start of training and on day 5 before and after training trials. For these trials, time spent in the target quadrant (where platform was positioned during training) was analysed. All measurements for swimming path were automatically recorded and computed by the video tracking system.

Isolation and culture of neural progenitor cells (NPCs)

Lewis rats expressing GFP [29] were kindly obtained from Prof. Kobayashi (Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Japan) and bred with female Lewis rats obtained from Charles River Laboratories, Germany. For the NPC isolation from GFP-expressing rat embryos, pregnant Lewis females were sacrificed by a lethal dose of pentobarbital (150 mg/kg, i.p.) on fetal day 16. Embryonic sacs were removed from the uterus and the embryos transferred into ice cold dissection medium consisting of HBSS (Sigma-Aldrich, H6648, Switzerland), antibiotic-antimycotic 1x (Gibco, Thermo Fisher Scientific, 15240-096, USA) and 6 mg/ml 45% glucose (Sigma-Aldrich, G8769, Switzerland). GFP-expressing embryos were identified using a UV lamp, brains removed and the hippocampal tissue dissected as described in [30]. Using a sterile scalpel, the tissue was cut into small pieces and incubated in 0.05% trypsin/EDTA (Bioswisstech, L2143, Switzerland) for 10 minutes at 37°C. After adding an equal volume of trypsin inhibitor 1 mg/ml (Sigma-Aldrich, T6522, Switzerland) and centrifugation for 5 minutes at 220g, the pellet was resuspended in 0.2 ml serum-free medium (SFM:

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DMEM/F12 + L-Glutamine (Gibco, Thermo Fisher Scientific, 11320-074, USA), 15mM HEPES (own production)). By gently pipetting up and down with differently sized pipet tips, cells were then mechanically dissociated. The obtained single cell suspension was cultured at a concentration of $5x10^4$ cells/ml in poly-HEMA-coated flasks (Sigma-Aldrich, P3932, Switzerland) in SFM supplemented with 20 µl/ml B27 50x (Thermo Fisher Scientific, 17504-044, USA), 10 ng/ml basic fibroblast growth factor (PeproTech, 100-18B, United Kingdom) and 10 ng/ml epidermal growth factor (PeproTech, AF-100-15, United Kingdom). Cells were grown to neurospheres at 37°C in 5% CO2 with medium changed every 2-3 days. After 6-8 days of culture, spheres were dissociated chemically and mechanically into single cells as described above and concentrated to $5x10^7$ cells/ml in phosphate-buffered saline (PBS) for transplantation.

Stereotaxic Surgery

One week after cardiac arrest and resuscitation or sham surgery, GFP-expressing NPCs were transplanted into both hippocampi. Animals were anesthetized with 0.15 mg/kg medetomidine (Domitor, Provet AG, Switzerland), 2 mg/kg midazolam (Dormicum, Roche, Switzerland) and 5 µg/kg fentanyl (Fentanyl Sintetica, Sintetica SA, Switzerland) administered s.c. After surgical anesthetic depth was reached, 20 µl lidocaine 1% (Lidocain HCl, Bichsel AG, Switzerland) was administered s.c. for local anesthesia, and the rats were fixed in a stereotaxic apparatus (Kopf Instruments, Model 902 Dual Small Animal Stereotaxic Instruments, USA). Oxygen was supplied via a nose mask throughout surgery, and body temperature was kept at 37°C with a temperature-controlled heating mat. After exposing the skull by a midline incision and removal of skin, muscle and periosteum, two holes were drilled at the following coordinates starting from bregma: AP: -4.0 mm, L:+/-2.3 mm, -2.7 mm (The Rat Brain In Stereotactic Coordinates, G. Paxinos & C. Watson, Academic Press 1988, 4th Edition). The NPC suspension (5x10^7 cells/ml) was injected into both hippocampi, 2µl each, using a 10µl Hamilton syringe. Drill holes were sealed with bone wax (Ethicon LLC, USA) and the incision sewed. 2 mg/kg Ropivacain (ROPIvacain Fresenius, Fresenius Kabi, Switzerland) was injected s.c. for local anaesthesia after surgery, and 100 mg/kg ceftriaxone (Rocephine, Roche, Switzerland) s.c. for postoperative prophylaxis. Anaesthesia was then reversed with 0.75 mg/kg atipamezole (Revertor, Virbac AG, Switzerland), 0.2 mg/kg flumazenil (Flumazénil Labatec, Labatec Pharma AG,

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Switzerland) and 0.05 mg/kg buprenorphine (Temgesic, Reckitt Benckiser AG, Switzerland) administered s.c., and animals kept on a heating mat until fully awake.

Histological assessments

For histological damage assessment, animals were sacrificed at given time points by a pentobarbital (Esconarkon, Streuli Pharma, Switzerland) overdose (150mg/kg, i.p.). After perfusion with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), brains were removed and further fixed in 4% PFA over-night at 4°C. They were then transferred to a 18% sucrose in PBS solution and stored at 4°C until further processed.

Assessment of degenerating neurons:

Coronal cryosections of the hippocampus (10 µm) obtained by systematic sampling (every 45th section collected) were stained with Fluoro-Jade B (Merck, AG310) to identify degenerating neurons 1 week after surgery. Briefly, cryosections were hydrated in 94% and 70% ethanol for 3 and 1 minutes respectively and after rinsing in deionized water they were incubated in a 0.06% potassium permanganate solution for 15 minutes. After rinsing, sections were stained with a 0.001% Fluoro-jade B (Merck, AG310, Germany) solution for 30 minutes in the dark. This solution was freshly prepared from a 0.01% stock solution in dH_20 and 0.1% acetic acid. After rinsing 3 times, sections were stained for DAPI and dried in the dark. Before embedding in DPX (Merck, 06522), they were cleared in xylene twice for 2 minutes. From every brain, 5 sections spanning the hippocampus of both hemispheres were imaged with a Zeiss fluorescent microscope (AxioImager M4, Zeiss, Germany) and 5 pictures were taken from every CA1 segment with a 200x magnification. Thus, a total of 50 pictures were quantified per animal. Using ImageJ 1.45I software (Wayne Rasband, National Institute of Health, USA), the CA1 segment length was determined and the Fluoro-Jade B positive cells were quantified. Blinding of the investigator was not performed, given the obvious difference between cardiac arrest and sham groups, i.e. the massive presence of degenerating cells in cardiac arrest animals only.

Assessment of surviving neurons:

For quantification of surviving neurons, hippocampal cryosections sampled using an identical protocol as above were stained with NeuN antibody (neuronal nuclear antigen, FOX3 antibody Merck ABN51) (see below for protocol), specific for mature neurons, and the whole CA1 segment was imaged (100x magnification) for a total of 50 pictures per

animal. Quantification of neurons was performed using ImageJ software as described above. The investigator conducting the quantification was blinded to the animal groups and time points.

Assessment of transplanted cells:

For histological analysis of brains after cell transplantation every 8th coronal cryosections (10µm) was collected (approx 40 sections / animal) and stained with different antibodies. See below for antibodies and protocols. For the quantification of animals with grafted cells in the CA1 segment, every section was checked for GFP expressing cells in this area, thereby only cells not located at the injection site or within the migration path towards the dentate gyrus were considered. This evaluation was performed blinded to the animal groups.

Immunofluorescent staining:

Immunofluorescent staining was performed with a Shandon Sequenza staining rack (Thermo Fisher Scientific, USA). Sections were permeabilized for 5 minutes with 0.1% Triton X-100 in PBS and then blocked with 0.01% Triton X-100 + 2% BSA for 1 hour at room temperature. Primary antibodies (table 1) were diluted in the same solution and added 1 hour at 37°C or overnight at 4°C. Slides were then rinsed 3 times with PBS and secondary antibodies (table 1) were added for 2 hours at room temperature. After rinsing again 3 times with PBS, samples were mounted using Fluoroshield containing DAPI (Sigma-Aldrich, F6057, Switzerland).

Statistical analysis

Statistical analyses were performed with GraphPad Prism 7 (GraphPad Software Inc, USA). If not stated otherwise, results are presented as mean ± standard deviations. An unpaired Student *t* test was used to compare data between two groups, and a two-way ANOVA was performed to analyse repeated measures of cardiac arrest versus sham operated animals (probe trials of Morris water maze). The association between the presence of transplanted cells within the CA1 segment and whether the animals underwent cardiac arrest or not was determined using Fisher's exact test. A two-sided p value of < 0.05 was considered statistically significant, with $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

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Results

Survival after cardiac arrest

Cardiac arrest was induced in a total of 94 animals. All but one animal could be resuscitated. Return of spontaneous circulation (ROSC) was achieved within 60.5 ± 30.3 seconds, and an average dose of 17.1 ±6.2 µg/kg adrenaline was injected. Of the resuscitated animals, 9 died spontaneously after ROSC or extubation. Ten animals had to be euthanized during the following 24 hours, due to insufficient recovery after surgery, leading to an overall survival rate of 78.7%.

After surgery, all animals lost weight and while the cardiac arrest groups started to regain weight after 4-5 days, the sham operated animals regained weight by the second day.

CA1 degeneration following cardiac arrest

Fluoro-Jade B (FJ) staining 1 week after surgery revealed a massive increase in neuronal degeneration within the CA1 pyramidal cell layer after global brain ischemia (98.7 ± 9.4 FJ-positive cells/mm, n = 4) compared to sham surgery (0.3 ± 0.2 FJ-positive cells/mm, n = 5; $p < 0.0001$) (figure 1A). FJ-positive cells were found exclusively in the CA1 segment of the hippocampus, while none were found in the CA3 segment (supplementary figure 1A). To investigate whether this cell loss was permanent, we further quantified surviving neurons 1, 4 and 6 weeks after surgery. Assessment of NeuN staining after 1 week confirmed the damage with a severely reduced number of mature neurons in the CA1 segment (73.5 ± 36.4 NeuN-positive cells/mm, n = 9) compared to sham (186.5 ± 7.9 NeuN-positive cells/mm, n=2, $p<0.01$) (figure 1B-C). The CA3 segment of ischemic animals were completely unaffected (suppl. figure 1B). Cell loss persisted 4 weeks after surgery with 86.1 ± 36.4 NeuN-positive cells/mm (cardiac arrest, n=8) vs 172.4 ± 1.0 NeuN-positive cells/mm (sham, n=2, $p<0.05$), and after 6 weeks: 62.5 ± 40.0 NeuN-positive cells/mm (cardiac arrest, n=9) vs 164.0 ± 22.5 NeuN-positive cells/mm (sham, n = 2, $p<0.01$). Overall, the number of surviving neurons in the CA1 segment after global brain ischemia did not evolve up to 6 weeks after the insult, and persisted at a stable low level (figure 1B).

Microglia accumulation shown by ionized calcium-binding adapter molecule 1 (Iba1) immunoreactive cells 5 days after injury [31] persisted until 4 weeks after surgery in the damaged area (figure 1E). The amoeboid-like morphology of microglia, characteristic for an activated state [32], was well demarcated from the surrounding healthy cortical tissue

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with strong ramification and long processes of resting microglia [32]. Further, glial fibrillary acidic protein (GFAP)-positive astrocytes displaying a reactive, hypertrophic phenotype [33], were abundant in the injured area (figure 1F) while astrocytes in unaffected sectors such as the dentate gyrus displayed a resting morphology.

Transient spatial memory deficits after cardiac arrest and resuscitation

After recovery from cardiac arrest and resuscitation, animals did not show significant locomotor impairments compared to controls, as determined in an open field test performed in the week preceding the behavioural experiments (suppl. figure 2), and confirmed by unaffected swimming velocity during Morris water maze test (suppl. figure 3).

Four weeks after surgery, learning ability was observed both in controls (sham, n = 10) and ischemic animals (cardiac arrest, n = 13), indicated by the decrease in time to find the platform over the 5 training days (figure 2A). Spatial memory performance was assessed by probe trials without platform. During the first probe trial on day 5, cardiac arrest animals spent significantly less time in the target quadrant (24.0 ± 6.5 % of the total time) compared to controls (32.2 ± 9.4 %, $p<0.05$), indicating a deficiency in memorizing the platform's location (figure 2B). This difference was still present during the second probe trial performed immediately after training, without reaching significance, indicating short-term memory deficits may have developed as well. Overall, ischemic animals exhibited an impaired memory performance compared to controls (Two-way ANOVA, $p<0.05$). Six weeks after cardiac arrest and resuscitation, learning ability of the two groups was comparable (figure 2C), and both groups spent a similar amount of time in the target quadrant during probe trials (figure 2D). In summary, we documented significant memory deficits 4 weeks after global brain ischemia, which could not be detected anymore 2 weeks later.

Transplanted NPCs survive up to 6 weeks and are increasingly found in CA1 segment after ischemia

We found viable cells up to 6 weeks after transplantation (figure 3). After 1 week, grafted cells were found in the CA1 segment of 40% of ischemic animals (n = 2/5), whereas none were detected in this region in controls (n = 0/5). One week later, 75% of injured animals presented GFP-expressing cells in the segment of interest (n = 6/8), compared to 16% of

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sham animals (n = 1/6). Together, in 62% of ischemic animals, GFP-expressing cells were found in the CA1 segment as compared to 9% for controls ($p<0.05$) (figure 3D), indicating an increased propensity of grafted cells to persist in the CA1 segment after injury. Six weeks after transplantation, GFP-expressing cells were found in 78% of cardiac arrest animals (n = 7/9). Numerous GFP-expressing cells displayed a neuronal morphology in the CA1 segment (figure 3B, F and suppl. figure 4B). Especially after 6 weeks, these cells were found to align along the pyramidal cell layer in some animals (figure 3E and suppl. figure 4C). In several instances transplanted cells also accumulated along the corpus callosum with migration of individual cells towards the CA1 segment (suppl. figure 4A-B). However, more cells still migrated toward their endogenous niche at the dentate gyrus where they accumulated along the granular cell layer in both ischemic (figure 3A) and control animals (figure 3C).

To identify an underlying molecular mechanism sustaining the observed persistence of cells in the injured CA1 segment, the stromal cell-derived factor 1 / CXC chemokine receptor 4 pathway (SDF-1 / CXCR4) was investigated, known to be pivotal for the migration of neural stem cells towards injured brain regions [34]. One week after cardiac arrest, at the time point of NPC transplantation, microglia and astrocytes accumulating in the damaged CA1 segment expressed the chemotactic molecule SDF-1 (figure 4A-B). Further, its receptor CXCR4 was found to be expressed in cultured NPCs, at the time point of transplantation (figure 4C) as well as 2 weeks later in the brain parenchyma (figure 4D).

Six weeks after transplantation, we found a considerable number of GFP-positive cells expressing the mature neuron marker NeuN, particularly in cells located in the CA1 segment (figure 5A). Other grafted cells were either positive for the immature neuron marker βIII-tubulin (figure 5B) or for stem-and progenitor cell markers such as SRY-box transcription factor 2 (SOX2) (figure 5D) and vimentin (figure 5C). Interestingly, most GFP-expressing cells found in the granular layer of the dentate gyrus co-expressed SOX2, while most grafted cells located in the CA1 segment did not (figure 5D-E). None of the grafted cells were found to express glial markers (suppl. figure 5).

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Discussion

Extension of cardiac arrest time to 12 minutes

When compared with our previous study [35], extension of no-flow time from 10 to 12 minutes resulted in a similar rate of survival, time to ROSC and administration of adrenaline. However, the extent of histopathological damage, was not increased. We performed the quantification 2 days later, thus it cannot be excluded that we would have found more degenerating cells at day 5. Although in our protocol the no-flow time was significantly longer than the usual median of 6-8 minutes in cardiac arrest models, we reached a higher ROSC and survival rate than the median of 85% and 60% respectively [36].

Permanent loss of CA1 neurons following global brain ischemia

Persistency of neuronal loss in the CA1 segment is in contrast to other studies which showed regeneration of the damaged area following global brain ischemia [37-41]. It was speculated that new neurons originate from progenitor cells residing around the posterior periventricle which proliferate in response to injury, migrate towards the damaged area, differentiate and integrate in the existing circuit [37]. In some instances this repopulation was correlated to a recovery of previously detected learning and memory deficits [37,39,40]. However, these studies used a different model of global brain ischemia, induced by vessel occlusion that exclusively affects the brain. Contrarily, the present model of cardiac arrest and resuscitation leads to ischemia and reperfusion injury throughout the body, associated with a systemic inflammatory response [14]. These events may lead to a more severe and intricate brain damage, which could negatively impact the endogenous regenerative potential. Since some of the publications are long-term studies up to 4 months [40], we cannot exclude similar findings if the follow up time was extended. The majority of vessel occlusion studies first performed behavioral experiments 1-2 weeks after injury [37,39,40,42]. In our experimental model this is not feasible, since a longer recovery period is needed, as is the case in other cardiac arrest models [43]. Contradictory results generated in specific models of global brain ischemia have been reported before [44,45]. Thus, the experimental settings applied to induce global brain ischemia are critically defining the outcome, which makes direct comparisons between the models difficult.

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Transient memory deficits after cardiac arrest and resuscitation

The memory impairments observed at 4 weeks, were compensated by 6 weeks after cardiac arrest. Based on the massive hippocampal cell death, we expected more severe impairments in the water maze performance. The transient nature of the observed memory deficits is in accordance to several studies, which correlated it to endogenous regeneration provided by cell replenishment [37,39,40]. Here, this is unlikely, since no repopulation of the damaged area was found. The striking discrepancy between the histological damage and the moderate cognitive deficits observed could be explained by the involvement of non-hippocampal brain regions in spatial memory performance. These structures might be able to take over the role of the injured area, in accordance to the brain plasticity concept. Additionally, the neurofunctional impairments detected with the water maze paradigm might not exclusively be caused by the CA1 cell loss. This would imply that the water maze paradigm involves the activity of hippocampus-unrelated structures. Although both hypotheses are supported by numerous studies in animals and humans [46-51], they are often neglected when focusing on hippocampal dysfunction and its influence on learning and memory. Our difficulties to detect deficits at later time points might also be due to the failure of a too simple water maze protocol to detect differences between groups that get more subtle over time, as has been demonstrated by more challenging paradigms [42,43,49]. The protocols applied for behavioral assessment considerably influence the experiment's outcome, and might in part explain the differences of our results with other studies describing persisting memory deficits after ischemia [41,42].

We are aware that for the evaluation of a regenerative treatment not only histological analyses, but additional long-term functional outcome measures should be considered . However, the effect of cardiac arrest on long-term learning and memory behaviour has still not been investigated thoroughly enough in rodent models, and the few available studies are contradictory, observing both persistent [41,42] and transient [37,39,40] memory deficits. Additionally to learning and memory behaviour, sensorimotor analysis after cardiac arrest would be another functional outcome measure. However, in our previous work[52], we did not find any persistent sensorimotor deficits after 8 minutes of cardiac arrest, and the same applies to the present study. Nevertheless, the described

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experimental model represents a valuable tool for the evaluation of novel therapeutic strategies, in particular when aiming at an increased neuroregeneration, easily detectable by histological analysis, since no spontaneous repopulation of the damaged CA1 segment was observed.

Retention of transplanted neural progenitor cells in the damaged CA1 segment We observed viable cells up to 6 weeks after grafting and an increased propensity of the cells to persist in the CA1 segment in ischemic animals. It is known that following brain damage, endogenous or transplanted neural stem cells (NSCs) migrate to the injury site [53]. This mobilization might be induced by the inflammatory response occurring there. Immune cells, including astrocytes and microglia, produce attractants, which guide the NSCs toward their sources [34,54-56]. The same molecular mechanisms might be responsible for the observed accumulation of transplanted cells in the CA1 segment after cardiac arrest. Indeed, we could confirm this hypothesis in our study, by showing the presence of a large number of reactive SDF-1-positive glia in the damaged CA1 segment and the expression of its receptor CXCR4 by the transplanted cells suggesting a chemotactic-driven retention at the site of injury. However, this mechanism may compete with signals provided by the dentate gyrus, since we documented a large proportion of transplanted cells migrating to its granule cell layer, i.e. their endogenous niche. At the injury site, we documented expression of NeuN or βIII-tubulin in a considerable proportion of transplanted cells, confirming their potential to differentiate towards mature neurons. However, an important part of NPCs still displayed an undifferentiated phenotype, expressing Vimentin and SOX2. These cells have maintained their stem and progenitor identity, but may still influence the regenerative processes through immunomodulation and trophic support [57]. Interestingly, the majority of GFP-expressing cells found in the dentate gyrus expressed SOX2, indicating a stronger stimulus to retain their multipotency, as expected from their stem cell niche environment. Although located in the dentate gyrus and not directly in the damaged area, these cells could potentially have an effect on regeneration through trophic support.

Overall, the observed long-term survival, the SDF-1-driven retention at the damaged area and the differentiation capacity of transplanted NPCs underlines their potential in supporting endogenous regeneration by replacing lost cells.

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Limitations of this study include the use of healthy young male animals, which is in contrast to the population of cardiac arrest patients that is mostly composed of older people often affected by co-morbidities [58,59]. Additionally, we only assessed hippocampal damage although other brain areas may have been affected by ischemia. Given the lack of differences between sham and cardiac arrest animals at the planned time point we assessed neurofunctional outcome, we were not able to assess the functional effect of grafted NPCs.

Conclusion

We conclude that NPC transplantation after cardiac arrest is feasible, with documentation of their survival, persistence at the injured site, neuronal differentiation and therefore potential for cell replacement. Further, we confirmed two of the key consequences of cardiac arrest, loss of CA1 pyramidal neurons and early memory deficits in our experimental model. For studies focusing on cardiac arrest sequelae, our model of global brain ischemia might be more relevant than vessel occlusion models since it recapitulates more accurately the complex cascade of pathological processes occurring in patients.

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Author disclosure statement

No competing financial interests exist.

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References

1. Berdowski J, RA Berg, JG Tijssen and RW Koster. (2010). Global incidences of out-of-hospital cardiac arrest and survival rates: Systematic review of 67 prospective studies. Resuscitation 81:1479-87.
2. Madl C and M Holzer. (2004). Brain function after resuscitation from cardiac arrest. Curr Opin Crit Care 10:213-7.
3. Grubb NR, R O'Carroll, SM Cobbe, J Sirel and KAA Fox. (1996). Chronic Memory Impairments after OHCA. BMJ 313:143-6.
4. Moulaert VR, JA Verbunt, CM van Heugten and DT Wade. (2009). Cognitive impairments in survivors of out-of-hospital cardiac arrest: a systematic review. Resuscitation 80:297-305.
5. Schmidt-Kastner R and TF Freund. (1991). Selective vulnerability of the hippocampus in brain ischemia. Neuroscience 40:599-636.
6. Kirino T. (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 239:57-69.
7. Pulsinelli WA, JB Brierley and F Plum. (1982). Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11:491-8.
8. Nunn J and H Hodges. (1994). Cognitive deficits induced by global cerebral ischaemia: relationship to brain damage and reversal by transplants. Behav Brain Res 65:1-31.
9. Zola-Morgan S, LR Squire and DG Amaral. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 6:2950-67.
10. Bernard SA, TW Gray, MD Buist, BM Jones, W Silvester, G Gutteridge and K Smith. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl J Med 346:557-63.
11. (2002). Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 346:549-56.

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12. Nielsen N, J Wetterslev, T Cronberg, D Erlinge, Y Gasche, C Hassager, J Horn, J Hovdenes, J Kjaergaard, M Kuiper, T Pellis, P Stammet, M Wanscher, MP Wise, A Aneman, N Al-Subaie, S Boesgaard, J Bro-Jeppesen, I Brunetti, JF Bugge, CD Hingston, NP Juffermans, M Koopmans, L Kober, J Langorgen, G Lilja, JE Moller, M Rundgren, C Rylander, O Smid, C Werer, P Winkel and H Friberg. (2013). Targeted temperature management at 33 degrees C versus 36 degrees C after cardiac arrest. N Engl J Med 369:2197-206.

13. Donnino MW, LW Andersen, KM Berg, JC Reynolds, JP Nolan, PT Morley, E Lang, MN Cocchi, T Xanthos, CW Callaway and J Soar. (2016). Temperature Management After Cardiac Arrest: An Advisory Statement by the Advanced Life Support Task Force of the International Liaison Committee on Resuscitation and the American Heart Association Emergency Cardiovascular Care Committee and the Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation. Resuscitation 98:97-104.

14. Huang L, PM Applegate, JW Gatling, DB Mangus, J Zhang and RL Applegate. (2014). A systematic review of neuroprotective strategies after cardiac arrest: from bench to bedside (part II-comprehensive protection). Medical Gas Research 4:10-10.

15. Geocadin RG, MA Koenig, X Jia, RD Stevens and MA Peberdy. (2008). Management of brain injury after resuscitation from cardiac arrest. Neurol Clin 26:487-506, ix.

16. Nolan JP, RA Berg, S Bernard, BJ Bobrow, CW Callaway, T Cronberg, RW Koster, PJ Kudenchuk, G Nichol, GD Perkins, TD Rea, C Sandroni, J Soar, K Sunde and A Cariou. (2017). Intensive care medicine research agenda on cardiac arrest. Intensive Care Med 43:1282-1293.

17. Zhu SZ, V Szeto, MH Bao, HS Sun and ZP Feng. (2018). Pharmacological approaches promoting stem cell-based therapy following ischemic stroke insults. Acta Pharmacol Sin 39:695-712.

18. Janowski M, DC Wagner and J Boltze. (2015). Stem Cell-Based Tissue Replacement After Stroke: Factual Necessity or Notorious Fiction? Stroke 46:2354-63.

19. Ding DC, CH Lin, WC Shyu and SZ Lin. (2013). Neural stem cells and stroke. Cell Transplant 22:619-30.

20. Leong KH, LL Zhou, QM Lin, P Wang, L Yao and ZT Huang. (2016). Therapeutic effects of various methods of MSC transplantation on cerebral resuscitation following cardiac arrest in rats. Mol Med Rep 13:3043-51.

21. Wang T, W Tang, S Sun, T Xu, H Wang, J Guan, Z Huang and MH Weil. (2008). Intravenous infusion of bone marrow mesenchymal stem cells improves brain function after resuscitation from cardiac arrest. Crit Care Med 36:S486-91.

22. Hodges H, P Sowinski, P Fleming, TR Kershaw, JD Sinden, BS Meldrum and JA G. (1996). Contrasting effects of fetal CA1 and CA3 hippocampal grafts on deficits in spatial learning and working memory induced by global cerebral ischaemia in rats. Neuroscience 72:959-988.

23. Chu K, M Kim, SW Jeong, SU Kim and BW Yoon. (2003). Human neural stem cells can migrate, differentiate, and integrate after intravenous transplantation in adult rats with transient forebrain ischemia. Neurosci Lett 343:129-33.

24. Chung TN, JH Kim, BY Choi, SP Chung, SW Kwon and SW Suh. (2015). Adipose-derived mesenchymal stem cells reduce neuronal death after transient global cerebral ischemia through prevention of blood-brain barrier disruption and endothelial damage. Stem Cells Transl Med 4:178-85.

25. Sinden JD, F Rashid-Doubell, TR Kershaw, A Nelson, A Chadwick, PS Jat, MD Noble, H Hodges and JA Gray. (1997). Recovery of spatial learning by grafts of a conditionally immortalized hippocampal neuroepithelial cell line into the ischaemia-lesioned hippocampus. Neuroscience 81:599-608.

26. Hodges H, A Nelson, D Virley, TR Kershaw and JD Sinden. (1997). Cognitive deficits induced by global cerebral ischaemia: prospects for transplant therapy. Pharmacol Biochem Behav 56:763-80.

27. Frick T, D Springe, D Grandgirard, SL Leib and M Haenggi. (2016). An improved simple rat model for global cerebral ischaemia by induced cardiac arrest. Neurological Research 38:373-380.

28. Muri L, D Grandgirard, M Buri, M Perny and SL Leib. (2018). Combined effect of non-bacteriolytic antibiotic and inhibition of matrix metalloproteinases prevents brain injury and preserves learning, memory and hearing function in experimental paediatric pneumococcal meningitis. J Neuroinflammation 15:233.

29. Inoue H, I Ohsawa, T Murakami, A Kimura, Y Hakamata, Y Sato, T Kaneko, M Takahashi, T Okada, K Ozawa, J Francis, P Leone and E Kobayashi. (2005). Development of new inbred transgenic strains of rats with LacZ or GFP. Biochem Biophys Res Commun 329:288-95.

30. Fath T, YD Ke, P Gunning, J Gotz and LM Ittner. (2009). Primary support cultures of hippocampal and substantia nigra neurons. Nat Protoc 4:78-85.

31. Frick T, D Springe, D Grandgirard, SL Leib and M Haenggi. (2015). An improved simple rat model for global cerebral ischaemia by induced cardiac arrest. Neurol Res:1743132815Y0000000090.

32. Fernández-Arjona MdM, JM Grondona, P Granados-Durán, P Fernández-Llebrez and MD López-Ávalos. (2017). Microglia Morphological Categorization in a Rat Model of Neuroinflammation by Hierarchical Cluster and Principal Components Analysis. Frontiers in Cellular Neuroscience 11.

33. Lin RC, DF Matesic, M Marvin, RD McKay and O Brustle. (1995). Re-expression of the intermediate filament nestin in reactive astrocytes. Neurobiol Dis 2:79-85.

34. Imitola J, K Raddassi, KI Park, F-J Mueller, M Nieto, YD Teng, D Frenkel, J Li, RL Sidman, CA Walsh, EY Snyder and SJ Khoury. (2004). Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1α/CXC chemokine receptor 4 pathway. Proceedings of the National Academy of Sciences 101:18117-18122.

35. Glas M, T Frick, D Springe, A Putzu, P Zuercher, D Grandgirard, SL Leib, SM Jakob, J Takala and M Haenggi. (2018). Neuroprotection with the P53-Inhibitor Pifithrin-mu after Cardiac Arrest in a Rodent Model. Shock 49:229-234.

36. Vognsen M, BK Fabian-Jessing, N Secher, B Lofgren, C Dezfulian, LW Andersen and A Granfeldt. (2017). Contemporary animal models of cardiac arrest: A systematic review. Resuscitation 113:115-123.

37. Nakatomi H, T Kuriu, S Okabe, S-i Yamamoto, O Hatano, N Kawahara, A Tamura, T Kirino and M Nakafuku. (2002). Regeneration of Hippocampal Pyramidal Neurons after Ischemic Brain Injury by Recruitment of Endogenous Neural Progenitors. Cell 110:429-441.

38. Schmidt W and KG Reymann. (2002). Proliferating cells differentiate into neurons in the hippocampal CA1 region of gerbils after global cerebral ischemia. Neurosci Lett 334:153-6.

39. Bendel O, T Bueters, M von Euler, SO Ögren, J Sandin and G von Euler. (2005). Reappearance of Hippocampal CA1 Neurons after Ischemia is Associated with Recovery of Learning and Memory. Journal of Cerebral Blood Flow & Metabolism 25:1586-1595.

40. von Euler M, O Bendel, T Bueters, J Sandin and G von Euler. (2006). Profound but transient deficits in learning and memory after global ischemia using a novel water maze test. Behavioural Brain Research 166:204-210.

41. Langdon KD, S Granter-Button and D Corbett. (2008). Persistent behavioral impairments and neuroinflammation following global ischemia in the rat. Eur J Neurosci 28:2310-8.

42. Hartman RE, JM Lee, GJ Zipfel and DF Wozniak. (2005). Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. Brain Res 1043:48-56.

43. Weihs W, AM Warenits, F Ettl, IA Magnet, U Teubenbacher, A Hilpold, A Schober, C Testori, A Tiboldi, KT Mag, M Holzer, S Hoegler, A Janata and F Sterz. (2016). Reduced long-term memory in a rat model of 8 minutes ventricular fibrillation cardiac arrest: a pilot trial. BMC Vet Res 12:103.

44. Matchett GA, JB Calinisan, GC Matchett, RD Martin and JH Zhang. (2007). The effect of granulocyte-colony stimulating factor in global cerebral ischemia in rats. Brain research 1136:200-207.

45. Popp E, T Rabsahl, A Schneider, N Russ, F Spohr, P Vogel, BW Bottiger and P Teschendorf. (2009). Intracerebroventricular application of granulocyte colony-stimulating factor after cardiac arrest does not promote beneficial effects on cerebral recovery after cardiac arrest in rats. Resuscitation 80:478-83.

46. Eichenbaum H. (2000). A cortical-hippocampal system for declarative memory. Nat Rev Neurosci 1:41-50.

47. Pouzet B, WN Zhang, J Feldon and JN Rawlins. (2002). Hippocampal lesioned rats are able to learn a spatial position using non-spatial strategies. Behav Brain Res 133:279-91.

48. Gerlai RT, A McNamara, S Williams and HS Phillips. (2002). Hippocampal dysfunction and behavioral deficit in the water maze in mice: an unresolved issue? Brain Res Bull 57:3-9.

49. Mehla J, SH Deibel, J Faraji, T Saito, TC Saido, MH Mohajerani and RJ McDonald. (2019). Looking beyond the standard version of the Morris water task in the assessment of mouse models of cognitive deficits. Hippocampus 29:3-14.

50. Tulving E and HJ Markowitsch. (1997). Memory beyond the hippocampus. Curr Opin Neurobiol 7:209-16.

51. Grubb NR, KA Fox, K Smith, J Best, A Blane, KP Ebmeier, MF Glabus and RE O'Carroll. (2000). Memory impairment in out-of-hospital cardiac arrest survivors is associated with global reduction in brain volume, not focal hippocampal injury. Stroke 31:1509-14.

52. Zuercher P, D Springe, D Grandgirard, SL Leib, M Grossholz, S Jakob, J Takala and M Haenggi. (2016). A randomized trial of the effects of the noble gases helium and argon on neuroprotection in a rodent cardiac arrest model. BMC Neurol 16:43.

53. Garzon-Muvdi T and A Quinones-Hinojosa. (2009). Neural stem cell niches and homing: recruitment and integration into functional tissues. ILAR J 51:3-23.

54. Gonzalez R, MH Hamblin and JP Lee. (2016). Neural Stem Cell Transplantation and CNS Diseases. CNS Neurol Disord Drug Targets 15:881-886.

55. Merino JJ, V Bellver-Landete, MJ Oset-Gasque and B Cubelos. (2015). CXCR4/CXCR7 molecular involvement in neuronal and neural progenitor migration: focus in CNS repair. J Cell Physiol 230:27-42.

56. Belmadani A, PB Tran, D Ren and RJ Miller. (2006). Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:3182-3191.

57. Martino G and S Pluchino. (2006). The therapeutic potential of neural stem cells. Nature Reviews Neuroscience 7:395-406.

58. Nielsen N, J Wetterslev, T Cronberg, D Erlinge, Y Gasche, C Hassager, J Horn, J Hovdenes, J Kjaergaard, M Kuiper, T Pellis, P Stammet, M Wanscher, MP Wise, A Aneman, N Al-Subaie, S Boesgaard, J Bro-Jeppesen, I Brunetti, JF Bugge, CD Hingston, NP Juffermans, M Koopmans, L Kober, J Langorgen, G Lilja, JE Moller, M Rundgren, C Rylander, O Smid, C Werer, P Winkel, H Friberg and TTMT Investigators. (2013). Targeted temperature management at 33 degrees C versus 36 degrees C after cardiac arrest. N Engl J Med 369:2197-206.

59. Hirlekar G, M Jonsson, T Karlsson, J Hollenberg, P Albertsson and J Herlitz. (2018). Comorbidity and survival in out-of-hospital cardiac arrest. Resuscitation 133:118-123.

Table 1: Antibodies used in the study.

Primary antibody	Species	Dilution	Company
FOX3/NeuN	rabbit	1:1000	Merck, Germany, ABN51
SOX2	rabbit	1:500	Abcam, UK, ab97959
Iba1	rabbit	1:500	Wako, Japan, 019-19741
Olig2	rabbit	1:1000	Abcam, UK, ab109186
CXCR4	rabbit	1:100	Abcam, UK, ab2074
GFAP	mouse	1:500	Merck, Germany, MAB360
βIII-tubulin	mouse	1:1000	Promega, USA, G712A
Vimentin	mouse	1:100	Sigma Aldrich, Switzerland, V6630
SDF-1, APC conjugated	mouse	1:100	R & D Systems, USA, IC350A

Secondary antibody	Species	Dilution	Company
anti-rabbit Cy3	goat	1:1000	Jackson Immuno Research, UK, Cy3 111-165-144
anti-rabbit AF647	goat	1:500	Thermo Fisher Scientific, USA, A-21245
anti-mouse Cy3	donkey	1:1000	Jackson Immuno Research, UK, Cy3 715-165-151

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Figure legends

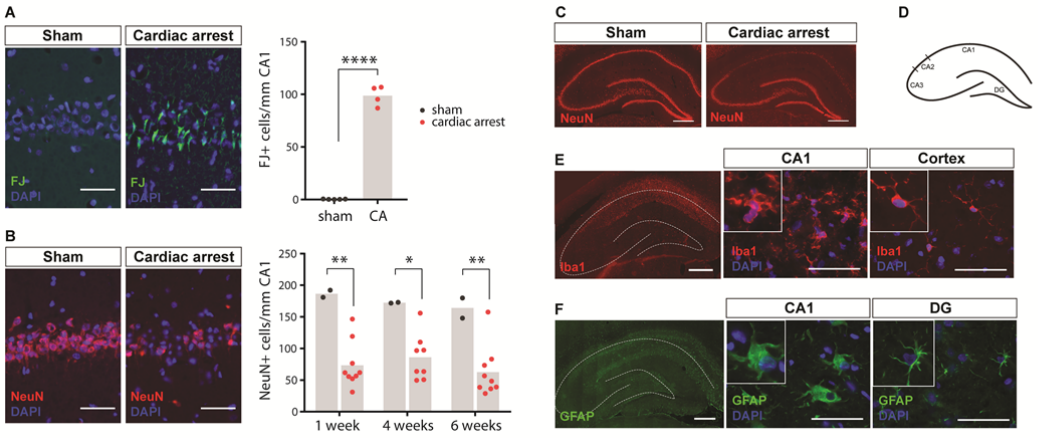

Figure 1: Histological damage assessment after cardiac arrest and resuscitation. Fluoro-jade B staining (FJ) 1 week after surgery, revealed the presence of degenerating neurons exclusively in the CA1 segment of cardiac arrest animals (CA) (A). Immunofluorescent staining for NeuN 1 week after resuscitation, confirmed the loss of mature neurons in the CA1 pyramidal layer after ischemia (B-C). Quantification of NeuN expressing neurons in CA1 pyramidal layer 1, 4 and 6 weeks after surgery (B). Overview of the whole hippocampus 1 week after cardiac arrest and resuscitation or sham surgery, stained for NeuN (C). Schematic representation of the main hippocampal structures *cornu ammonis* 1-3 (CA1-3) and dentate gyrus (DG) (C). Immunofluorescent staining for glial cell markers revealed a strong inflammatory response in the damaged CA1 segment 4 weeks after the insult (E-F). Iba1 expressing microglia (E) and GFAP positive astrocytes (F) were numerously present, and exhibited a reactive phenotype, in contrast to unaffected areas (cortex and DG). Scale bars 50 µm, except (C) and (E-F, whole hippocampus) 500 µm.

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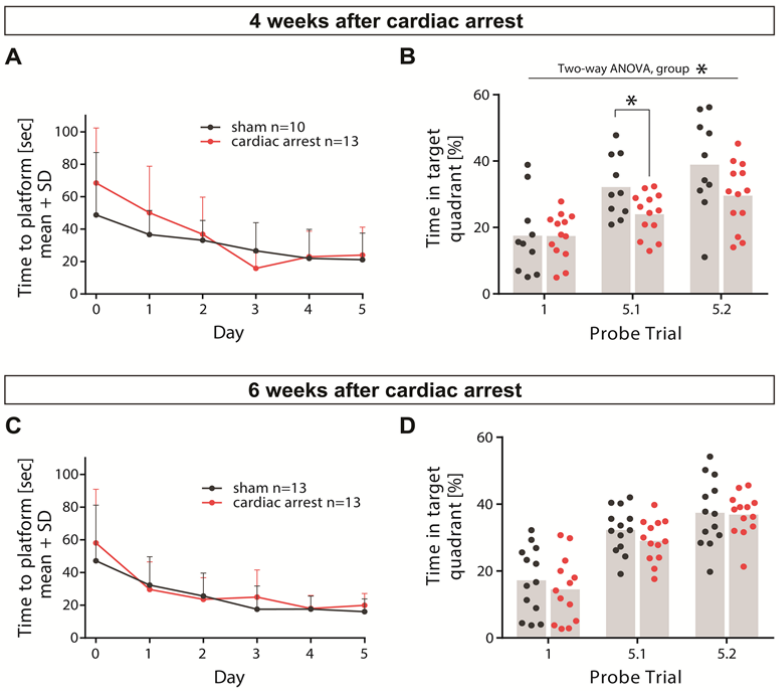

Figure 2: Neurofunctional assessment 4 and 6 weeks after surgery using Morris water maze. Learning performance of ischemic and control animals was similar at both time points, as indicated by the steady decrease in time to find the platform over the 5 training days (A, C). Day 0 represents the first training trial on day 1. Four weeks after cardiac arrest and resuscitation, animals showed significant memory impairments compared to controls (B). During both probe trials on day 5 (5.1 and 5.2) they spent less time in the target quadrant, where the platform was located during training, although the difference reached significance only in the first trial. Overall, cardiac arrest animals performed significantly worse than controls, as assessed by a two-way ANOVA. Six weeks after surgery, the difference in memory performance could not be detected anymore (D).

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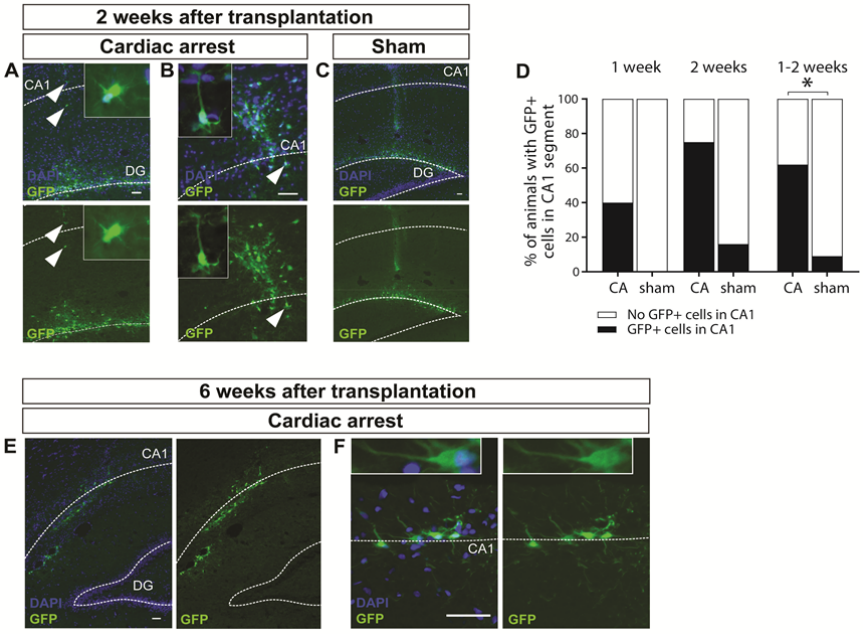

Figure 3: Location of GFP-expressing cells 2 and 6 weeks after transplantation. Grafted cells were found to accumulate along the granular layer of the dentate gyrus (DG) in both ischemic animals (A) as well as controls (C). However, in case of a damaged CA1 segment, GFP-expressing cells showed a stronger tendency to persist in injured area (A, B, E, F). In controls, grafted cells were only found in the CA1 layer during their migration towards the ventrally located dentate gyrus (C). Quantification showed that the probability of finding transplanted cells in the CA1 segment was significantly higher in cardiac arrest animals compared to controls (D). Scale bars 50 µm.

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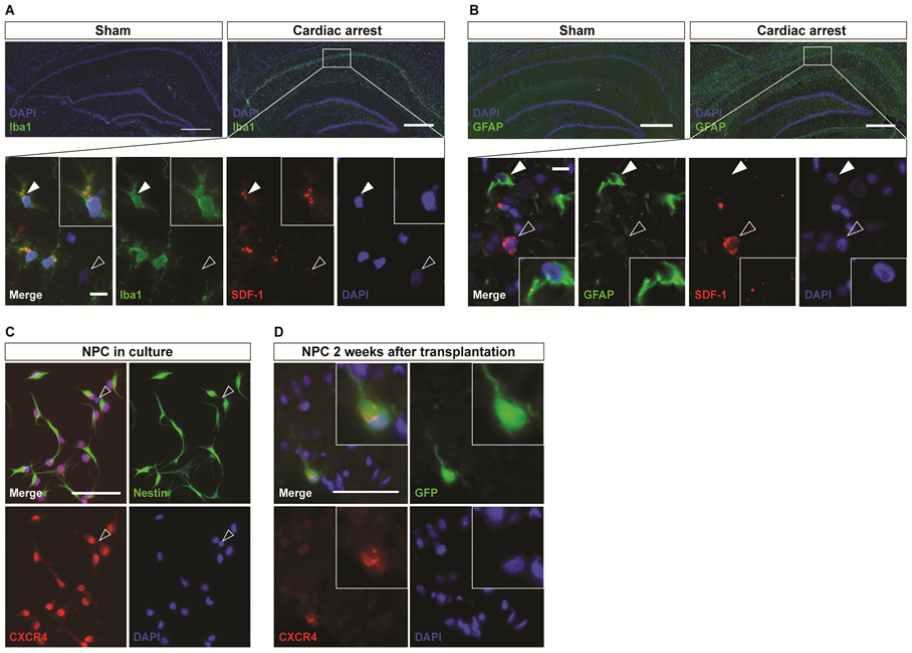

Figure 4: Expression of the chemotaxis molecule SDF-1 and its receptor CXCR4 by glia cells and transplanted NPCs respectively. One week after cardiac arrest, Iba1-positive microglia (A) and GFAP-positive astrocytes (B) accumulating in the damaged CA1 segment expressed SDF-1, while in sham animals almost no glia cells were found in the hippocampus. Other SDF-1 expressing cells (blank arrowheads) might be unstained glia cells or neurons, also known to express the chemokine [59]. Cultured nestin-positive NPCs expressed CXCR4 at the time point of transplantation (C), while nestin-negative cells (glia or differentiated neurons) did not (blank arrowhead). Two weeks after transplantation GFP-expressing NPCs were still positive for CXCR4 (D). Scale bars 500 µm (A-B, whole hippocampus), 10 µm (A-B, insets) and 50 µm (C-D).

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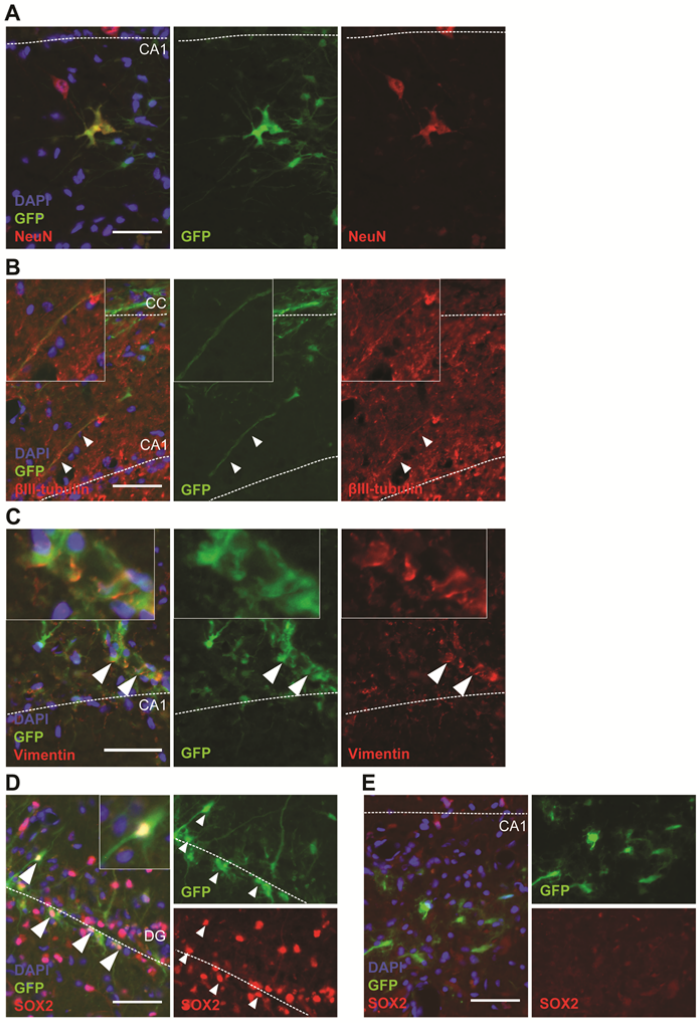

Figure 5: Phenotypic characterization of GFP-expressing cells 6 weeks after transplantation in cardiac arrest animals. A considerable number of grafted cells were found to express the mature neuron marker NeuN (A), while others were positive for βIII-tubulin, specific for immature neurons, (B). Further, some grafted cells remained positive for stem and progenitor cell markers, such as Vimentin (C) or SOX2 (D), the latter being expressed mainly by cells located in the dentate gyrus (D), and less by cells in the CA1 segment (E). Scale bars 50µm.

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Supplementary material

Induction of cardiac arrest and resuscitation

Anaesthesia and preparation for surgery:

Animals were prepared for surgery as previously described [1]. Briefly, anesthesia was induced with 5% sevoflurane (Sevorane, Abbott, Switzerland), and supplemented with fentanyl 20 µg/kg, i.p. (Fentanyl Sintetica, Sintetica SA, Switzerland). During surgery, anesthesia was maintained with 2.5-4% sevoflurane. Orotracheal intubation was performed with a 2.0 mm diameter angiocath (Venflon, BD, Germany) under direct vision, and the tube position was confirmed by capnography (Datex S/5 Anaesthesia Monitor, GE, Helsinki, Finland). The animals were ventilated with a pressure-controlled, time-cycled ventilator (KTR-5, Hugo-Sachs Elektronik, March, Germany). The initial ventilator settings were 50 breaths per minutes, 30% oxygen/air mix, an inspiratory pressure of 12 cmH2O and a PEEP (positive end-expiratory pressure) of 3 mmH2O. The ventilation was adapted after a first blood gas analysis to keep arterial p(CO_2) in the normal range (35-40 mmHg). A thermometer was placed in the esophagus and the animal's core temperature was kept at 36–37°C. This was achieved with a combination of a custom-made operation table, which was perfused with water at a temperature of 29-31°C, and an infrared heating lamp. A simple heated surgical table would lead to paraplegia during cardiac arrest because the heat produced by the table is directly transferred to the spine, without convection by the circulation blood. The right femoral artery was surgically exposed and a polyethylene (PE) 50 catheter for blood pressure monitoring and blood sampling was inserted. Another PE 50 catheter for drug administration was inserted into the right jugular vein. Recordings of blood pressure and temperature were performed with a standard anesthesia monitor (Datex S/5 Anaesthesia Monitor, GE Healthcare, Finnland).

Cardiac arrest and resuscitation:

Cardiac arrest and resuscitation were performed as previously described [1], but with extended no-flow time from 10 to 12 minutes. Animals were paralyzed with rocuronium bromide 10 mg/kg, i.v. (Esmeron, MSD Merck Sharp & Dhome AG, Switzerland) in order to suppress agonal breathing, and sevoflurane stopped. Cardiac arrest was induced by injecting i.v. 1 ml of a mixture of potassium chloride (KCl) and esmolol (0.5 ml of 2 mmol/ml KCl + 7.5 ml of esmolol 10 mg/ml, resulting in 0.125 mmol KCl and 9.375 mg

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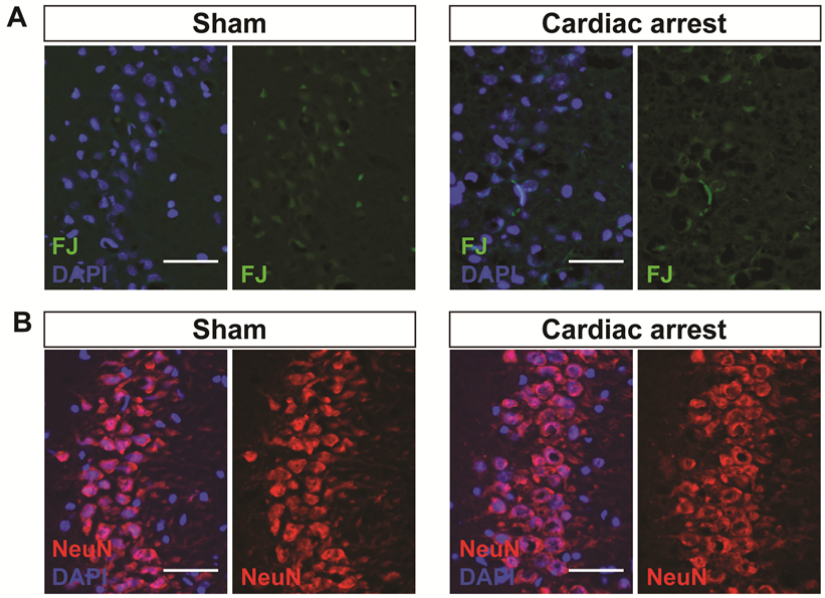
esmolol per animal) (Esmolol-Orpha, OrPha Swiss GmbH, Switzerland; Kalium Chloratum Sintetica, Sintetica SA, Switzerland), followed by 0.2 ml of lower concentrated KCl solution (0.1 ml of 2 mmol/ml KCl + 9.9 ml distilled water) (Aqua ad injectabilia Fresenius, Fresenius Kabi, Switzerland). Ventilation was disconnected and the heating lamp turned off. Cardiac arrest was indicated as a drop of arterial blood pressure below 15 mmHg. At 11'30'', the heating lamp was turned on and ventilation was resumed with 100% oxygen. The respiratory rate was set to 60 breaths per minute (bpm) and the initial five breaths were given with an inspiratory pressure of 20 cmH2O to recruit collapsed alveoli, then the pressure was reduced again to 12 cmH2O. At 12'00'', manual metronome-guided chest compressions with two fingers at a rate of 220/min were started while holding the animal with the other hand. The chest compressions were always made by the same operator (MH). Simultaneously, diluted adrenaline 15 µg/kg (Adrenalin Sintetica, Sintetica AG, Switzerland) and calcium 0.056mmol/kg (Calcium-Sandoz, Sandoz Pharmaceuticals AG, Switzerland) were injected i.v., separated by 0.1 ml of normal saline. If return of spontaneous circulation (ROSC) was not achieved within 60 seconds, additional adrenaline boli (5 µg/kg) were administered every 30 seconds until ROSC. Calcium was repeated after 2 minutes of attempted resuscitation. ROSC was defined as regular cardiac activity >200/min with a mean arterial pressure of ≥60 mmHg, sustained for at least 20 seconds. If ROSC was not achieved within 4 minutes, no further resuscitation attempts were made.
In control animals (sham group) no cardiac arrest was induced. Following catheter placement and rocuronium bromide injection, ventilation was maintained and after 12 minutes postoperative care was started.

Postoperative care:

After successful resuscitation, ventilation rate and pressure were increased to 70/min and 20 cmH_2O for 2 minutes, the pressure was reduced to 16 cmH_2O and further adapted after an arterial blood gas analysis. Arterial blood gas analyses were performed 5 and 20 minutes after ROSC, and ventilation adapted accordingly. Animals received amoxicillin 50 mg/kg (Clamoxyl, GlaxoSmithKline AG, Switzerland) for postoperative prophylaxis and fentanyl 20 µg/kg for analgesia, both i.m. After the last blood gas analysis, catheters were removed and wounds closed. Sevoflurane was added at a minimal dose to avoid self-extubation (0.6 to max 0.8% in cardiac arrest animals and 2.5% in sham operated animals),

and animals received fentanyl 20 µg/kg 1 and 2 hours post ROSC, s.c. For longer lasting analgesia, buprenorphine 20µg/kg s.c. (Temgesic, Reckitt Benckiser AG, Switzerland) was administered 3 hours post ROSC, before weaning from mechanical ventilation 1 hour later. After extubation, animals were placed in single cages for recovery. Moist food (Emeraid IC Omnivore, Emeraid LLC, USA) and hydrogel were provided for 2-3 days. General health, including weight, and neurobehavior, including motor activity, were assessed daily according to a standardized scoring system. If the minimum required score was not reached animals were euthanized. After surgery, all animals lost weight and while the cardiac arrest groups started to regain weight after 4-5 days, the sham operated animals regained weight by the second day. As soon as the animal's constitution allowed it, they were transferred to shared cages, usually 5-7 days after surgery

Supplementary figure legends



Suppl. figure 1: Histological damage assessment after cardiac arrest and resuscitation. No Fluoro-jade B (FJ) positive degenerating neurons were found in the CA3 segment of sham or cardiac arrest animals (A). CA3 integrity was confirmed in both groups by NeuN staining (B).

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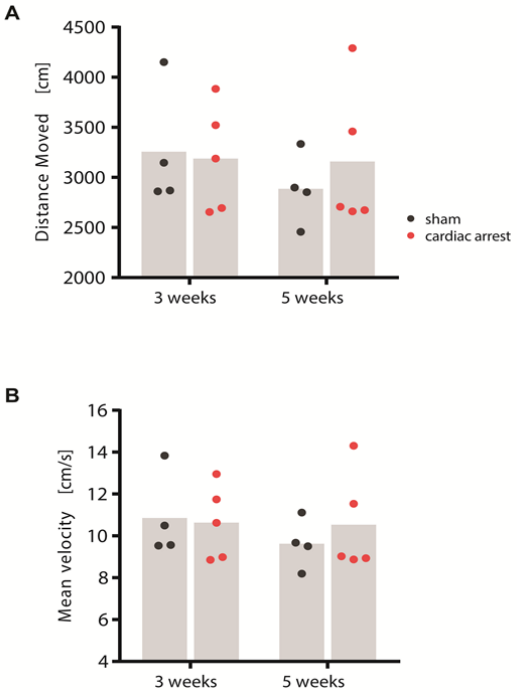

Suppl. figure 2: **Locomotor activity testing by open field test 3 and 5 weeks after cardiac arrest and resuscitation.** Open field tests were performed 1 week before animals would be assessed by Morris water maze, to ensure the absence of locomotor impairments. Total distance moved (A) and mean velocity (B) during the 5 minutes lasting trial from every tested animal. No difference was found between cardiac arrest and sham operated animals, as well as between the two time points.

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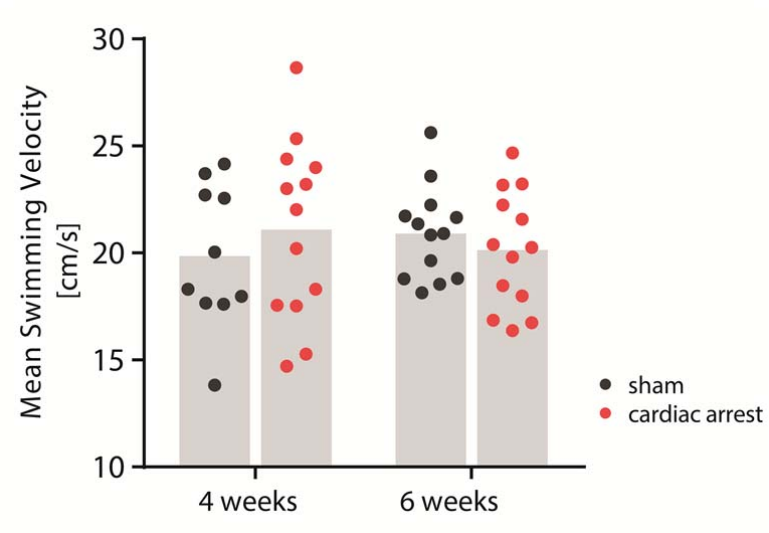

Suppl. figure 3: Swimming velocity during Morris water maze test 4 and 6 weeks after cardiac arrest and resuscitation. Mean swimming velocity during first probe trial on day 1. No difference was found between cardiac arrest and sham operated animals, as well as between the two time points.

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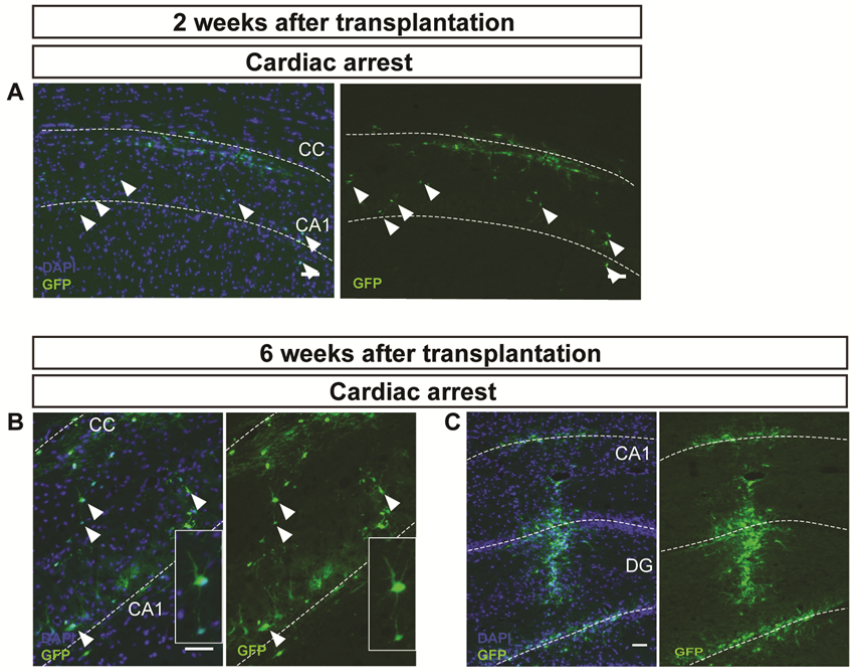

Suppl. figure 4: Location of GFP expressing cells 2 and 6 weeks after transplantation. In several animals we found an accumulation of cells along the corpus callosum (CC), and from there single cells migrated ventrally into the *cornu ammonis* field 1 (CA1) (A-B). Although a strong migratory stimulus towards the dentate gyrus (DG) was present also after cardiac arrest, a part of the cells persisted in the CA1 segment (C).Scale bar 50µm.

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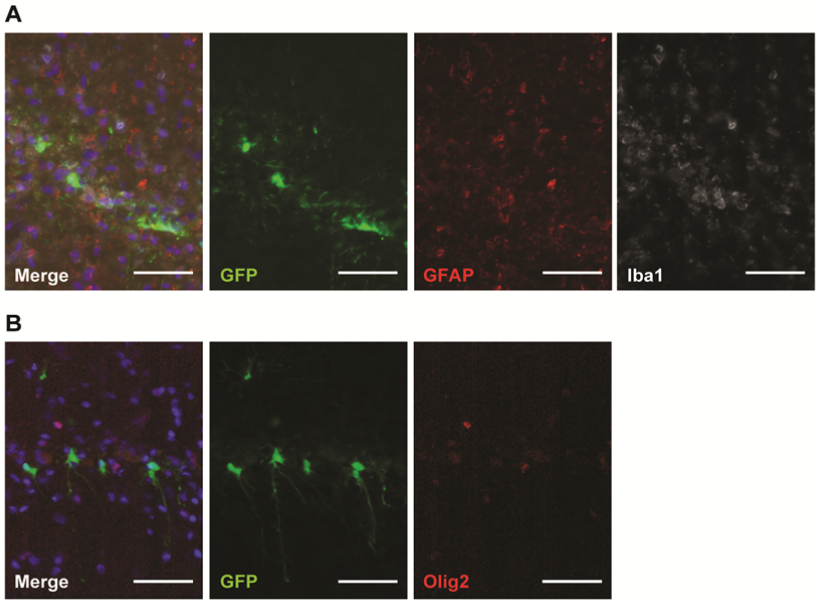

Suppl. figure 5: Immunofluorescent staining of transplanted cells for glial markers.

Immunofluorescent staining for astrocyte marker GFAP (red) and microglia marker Iba1 (grey) (A) as wells as oligodendrocyte marker Olig2 (red) (B). Transplanted cells express GFP (green). Scale bar 50µm.

1. Frick, T., et al., *An improved simple rat model for global cerebral ischaemia by induced cardiac arrest.* Neurological Research, 2016. **38**(4): p. 373-380.